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INVENTOR(S)		
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TITLE OF THE INVENTION (500 characters max)
Methods and Compositions for the Treatment of Gastrointestinal Disorders

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Respectfully submitted,

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PROVISIONAL APPLICATION FOR PATENT

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TITLE: METHODS AND COMPOSITIONS FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS

APPLICANT: MARK G. CURRIE

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Methods and Compositions for the Treatment of Gastrointestinal Disorders

TECHNICAL FIELD

This invention relates to methods and compositions for treating gastrointestinal disorders, obesity, congestive heart failure, benign prostatic hyperplasia and other disorders including hypertension and asthma.

BACKGROUND

Irritable bowel syndrome (IBS) is a common chronic disorder of the intestine that affects 20 to 60 million individuals in the US alone (Lehman Brothers, Global Healthcare-Irritable Bowel Syndrome Industry Update, September 1999). IBS is the most common disorder diagnosed by gastroenterologists (28% of patients examined) and accounts for 12% of visits to primary care physicians (Camilleri 2001 *Gastroenterology* 120:652-668). In the US, the economic impact of IBS is estimated at $25 billion annually, through direct costs of health care use and indirect costs of absenteeism from work (Talley 1995 Gastroenterology 109:1736-1741). Patients with IBS have three times more absenteeism from work and report a reduced quality of life. Sufferers may be unable or unwilling to attend social events, maintain employment, or travel even short distances (Drossman 1993 *Dig Dis Sci* 38:1569-1580). There is a tremendous unmet medical need in this population since few prescription options exist to treat IBS.

Patients with IBS suffer from abdominal pain and a disturbed bowel pattern. Three subgroups of IBS patients have been defined based on the predominant bowel habit: constipation-predominant (c-IBS), diarrhea-predominant (d-IBS) or alternating between the two (a-IBS). Estimates of individuals who suffer from c-IBS range from 20-50% of the IBS patients with 30% frequently cited. In contrast to the other two subgroups that have a similar gender ratio, c-IBS is more common in women (ratio of 3:1) (Talley et al. 1995 *Am J Epidemiol* 142:76-83).

The definition and diagnostic criteria for IBS have been formalized in the "Rome Criteria" (Drossman et al. 1999 *Gut* 45:Suppl II:1-81), which are well accepted in clinical practice. However, the complexity of symptoms has not been explained by anatomical abnormalities or metabolic changes. This has led to the classification of IBS as a functional GI disorder, which is diagnosed on the basis of the Rome criteria and limited evaluation to exclude organic disease(Ringel et al. 2001 *Annu Rev Me*d 52: 319-338). IBS is considered to be a "biopsychosocial" disorder resulting from a combination of three interacting mechanisms: altered bowel motility, an increased sensitivity of the intestine or colon to pain stimuli (visceral sensitivity) and psychosocial factors (Camilleri 2001 *Gastroenterology* 120:652-668). Recently, there has been increasing evidence for a role of inflammation in the etiology of IBS. Reports indicate that subsets of IBS patients have small but significant increases in colonic inflammatory and mast cells, increased inducible nitric oxide (NO) and synthase (iNOS) and altered expression of inflammatory cytokines (reviewed by Talley 2000, Medscape Coverage of DDW Week).

Guanylin is an intestinal peptide that stimulates chloride secretion. In humans, guanylin is produced initially as a 115 amino acid protein referred to as preproguanylin. The mature protein, which is believed to be the active form, has 15 amino acids. Guanylin is inactivated by cleavage by the serine protease, chymotrypsin, which is present in the gastrointestinal tract, and by a chymotrypsin-like enzyme that is present in the liver. Guanylin is an agonist of the transmembrane guanylate cyclase (GC-C) receptor. The GC-C receptor is present on the apical plasma membrane of enterocytes in intestinal tract and in other epithelia. Activation of the GC-C receptor by guanylin in the intestine increases cGMP levels. This increase in cGMP is believed to cause a decrease in water and sodium absorption and an increase in chloride and potassium ion secretion, leading to changes in intestinal fluid and electrolyte transport and increased intestinal motility. The intestinal GC-C receptor possesses an extracellular ligand binding region, a transmembrane region, an intracellular protein kinase-like region and a cyclase catalytic domain. Proposed functions for the GC-C receptor are fluid and electrolyte homeostasis, the regulation of epithelial cell proliferation and the induction of apoptosis (Shaibhubhai 2002 *Curr Opin Drug Dis Devel* 5:261-268).

Diarrhea is a common complication in HIV patients. It appears that diarrhea is particularly common in patients treated with protease inhibitors (Sherman et al. 2000 Clin Infect Dis 30:908). Prolonged diarrhea impacts quality of life and can contribute to weight loss, malnutrition, immunosuppression, poor drug absorption, non-compliance with therapy and mortality. Oat bran, psyllium, loperamide, calcium carbonate, pancrelipase, and SP-303 have been shown to have some beneficial effect on diarrhea associated with the use of HIV protease inhibitors (Sherman et al., supra). Bode et al. (AIDS 13:2595, 1999) state that the cause of HIV protease inhibitor associated diarrhea is unknown. Bode et al. also report that saquinavir, ritomavir, and nelfinavir, but not indinavir, impair the epithelial barrier in human HT-29/B6 cells.

SUMMARY

The present invention features compositions and related methods for treating IBS and other gastrointestinal disorders and conditions (e.g., gastrointestinal motility disorders, functional gastrointestinal disorders, gastroesophageal reflux disease (GERD), Crohn's disease, ulcerative colitis, inflammatory bowel disease, functional heartburn, dyspepsia (including functional dyspepsia or nonulcer dyspepsia), gastroparesis, chronic intestinal pseudo-obstruction (or colonic pseudoobstruction), and disorders and conditions associated with constipation, e.g., constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders.

The methods and the compositions in the invention relate to the administration of a compound that inhibits chymotrypsin activity. By interfering with the inactivation of guanylin in the intestinal tract, a chymotrypsin inhibitor can potentiate the action of naturally-occurring guanylin thereby increasing or regularizing intestinal motility. The invention also features the use of a chymotrypsin inhibitor in a combination therapy with therapeutically administered guanylin, a guanylin analogue or variant or some other treatment for a gastrointestinal disorder. In addition, the chymotrypsin inhibitor can be used to potentiate the action of the therapeutically administered guanylin.

The present invention also features compositions and related methods for treating obesity, congestive heart failure and benign prostatic hyperplasia (BPH).

Without being bound by any particular theory, in the case of IBS and other gastrointestinal disorders the compositions of the invention are useful because they can increase or regularize gastrointestinal motility by potentiating guanylin activity.

Without being bound by any particular theory, in the case of IBS and other gastrointestinal disorders the compositions of the invention are useful, in part, because they can decrease inflammation by potentiating guanylin activity.

Without being bound by any particular theory, in the case of IBS and other gastrointestinal disorders the compositions of the invention are also useful because they can decrease gastrointestinal pain or visceral pain by potentiating guanylin activity.

The invention features pharmaceutical compositions comprising certain compounds that are capable of reducing the activity of chymotrypsin, particularly the ability of chymotrypsin to inactivate guanylin by proteolytic cleavage. Also within the invention are pharmaceutical compositions comprising a chymotrypsin inhibitor as well as combination compositions comprising a chymotrypsin inhibitor and a second therapeutic agent, e.g., an agent for treating constipation or some other gastrointestinal disorder. Examples of a second therapeutic agent include: acid reducing agents such as proton pump inhibitors and H2 receptor blockers, pro-motility agents such as 5HT receptor agonists (e.g. Zelnorm®), anti-inflammatory agents, antispasmodics, antidepressants, analgesic agents (e.g., centrally-acting analgesic agents such as opioid receptor agonists, opioid receptor antagonists, and agents that treat gastrointestinal or visceral pain) and cGMP phosphodiesterase inhibitors (motapizone, zaprinast, and suldinac sulfone). Thus, for example, the pharmaceutical compositions can include an analgesic agent selected from the group consisting of: Ca channel blockers (e.g., ziconotide), 5HT receptor antagonists (for example 5HT3, 5HT4 and 5HT1 receptor antagonists), opioid receptor agonists (e.g., loperamide, fedotozine, and fentanyl, naloxone, naltrexone, methyl nalozone, nalmefene, cypridime, beta funaltrexamine, naloxonazine,

naltrindole, and nor-binaltorphimine, morphine, diphenyloxylate, enkephalin pentapeptide, and trimebutine), NK1 receptor antagonists (e.g., ezlopitant and SR-14033), CCK receptor agonists (e.g., loxiglumide), NK1 receptor antagonists, NK3 receptor antagonists (e.g., talnetant, osanetant (SR-142801)), norepinephrine-serotonin reuptake inhibitors (NSRI; e.g., milnacipran), vanilloid and cannabanoid receptor agonists (e.g., arvanil), sialorphin, sialorphin-related peptides comprising the amino acid sequence QHNPR (SEQ ID NO:) for example, VQHNPR (SEQ ID NO:); VRQHNPR (SEQ ID NO:); VRGQHNPR (SEQ ID NO:); VRGPQHNPR (SEQ ID NO:); VRGPRQHNPR (SEQ ID NO:); VRGPRRQHNPR (SEQ ID NO:); and RQHNPR (SEQ ID NO:), compounds or peptides that are inhibitors of neprilysin, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH_2; WO 01/019849 A1), loperamide, Tyr-Arg (kyotorphin), CCK receptor agonists (caerulein), conotoxin peptides, peptide analogs of thymulin, loxiglumide, dexloxiglumide (the R-isomer of loxiglumide) (WO 88/05774) and other analgesic peptides or compounds can be used with or linked to the inhibitors of the invention.

The invention includes methods for treating other disorders such as congestive heart failure and benign prostatic hyperplasia by administering a chymotrypsin inhibitor. Such agents can be used in combination with natriuretic peptides (e.g., atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide), a diuretic, or an inhibitor of angiotensin converting enzyme.

The invention features methods and compositions for increasing intestinal motility by potentiating the action of guanylin. Intestinal motility involves spontaneous coordinated distentions and contractions of the stomach, intestines, colon and rectum to move food through the gastrointestinal tract during the digestive process.

The invention features a therapeutic or prophylactic method comprising administering a composition comprising an inhibitor of chymotrypsin. For the treatment of gastrointestinal disorders, the inhibitor can be administered orally, by rectal suppository or parenterally.

In various embodiments, the patient is suffering from a gastrointestinal disorder; the patient is suffering from a disorder selected from the group consisting of: a gastrointestinal motility disorder, irritable bowel syndrome, a functional gastrointestinal disorder, gastroesophageal reflux disease, functional heartburn, dyspepsia, functional dyspepsia, nonulcer dyspepsia, gastroparesis, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, obesity, congestive heart failure, or benign prostatic hyperplasia. In another aspect, the invention features a method for treating a patient suffering from constipation, the method comprising administering a composition comprising, consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier. Clinically accepted criteria that define constipation range from the frequency of bowel movements, the consistency of feces and the ease of bowel movement. One common definition of constipation is less than three bowel movements per week. Other definitions include abnormally hard stools or defecation that requires excessive straining (Schiller 2001 *Aliment Pharmacol Ther* 15:749-763). Constipation may be idiopathic (functional constipation or slow transit constipation) or secondary to other causes including neurologic, metabolic or endocrine disorders. These disorders include diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple sclerosis, Parkinson's disease, spinal cord lesions, Neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung disease and cystic fibrosis. Constipation may also be the result of surgery or due to the use of drugs such as analgesics (like opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics.

In various embodiments, the constipation is associated with use of a therapeutic agent; the constipation is associated with a neuropathic disorder; the constipation is post-surgical constipation; the constipation is associated with a gastrointestinal disorder; the constipation is idiopathic (functional constipation or slow transit constipation); the constipation is associated with neuropathic, metabolic or endocrine disorder (e.g., diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple Sclerosis, Parkinson's disease, spinal cord lesions, neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung disease or cystic fibrosis). Constipation may also be the result of surgery or due

to the use of drugs such as analgesics (e.g., opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics.

In another aspect, the invention features a method for treating a patient suffering from a gastrointestinal disorder, the method comprising administering a composition comprising, consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier.

In various embodiments, the patient is suffering from a gastrointestinal disorder; the patient is suffering from a disorder selected from the group consisting of: a gastrointestinal motility disorder, irritable bowel syndrome, a functional gastrointestinal disorder, gastroesophageal reflux disease, functional heartburn, dyspepsia, functional dyspepsia, nonulcer dyspepsia, gastroparesis, chronic intestinal pseudo-obstruction, and colonic pseudo-obstruction.

In another aspect, the invention features a method for increasing gastrointestinal motility in a patient, the method comprising administering to the patient a composition comprising, consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier

In another aspect, the invention features a method for decreasing gastrointestinal pain or visceral pain in a patient, the method comprising administering to the patient a composition comprising, consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier composition comprising a chymotrypsin inhibitor.

In another aspect, the invention features a method for increasing the activity of an intestinal guanylate cyclase (GC-C) receptor in a patient, the method comprising administering to the patient a composition comprising, consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier.

In another aspect, the invention features a pharmaceutical composition comprising consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier. The composition can include a polymer that controls the release of the inhibitor. The composition can include a second agent, e.g., guanylin.

In another aspect, the invention features a method for treating obesity, the method comprising administering a pharmaceutical composition comprising consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier. The composition can be administered in combination with another agent for treatment of obesity, for example, sibutramine, phentermine, phendimetrazine, benzphetamine hydrochloride (Didrex), orlistat (Xenical), diethylpropion hydrochloride (Tenuate), fluoxetine (Prozac), bupropion, ephedra, chromium, garcinia cambogia, benzocaine, bladderwrack (focus vesiculosus), chitosan, nomame herba, galega (Goat's Rue, French Lilac), conjugated linoleic acid, L-carnitine, fiber (psyllium, plantago, guar fiber), caffeine, dehydroepiandrosterone, germander (teucrium chamaedrys), B-hydroxy-β-methylbutyrate, and pyruvate.

In another aspect, the invention features a method for treating congestive heart failure, the method comprising: administering to the patient a pharmaceutical composition comprising consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier. The composition can be administered in combination with another agent for treatment of congestive heart failure, for example, a natriuretic peptide such as atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide), a diuretic, or an inhibitor of angiotensin converting enzyme.

In another aspect, the invention features a method for treating benign prostatic hyperplasia, the method comprising: administering to the patient a pharmaceutical composition comprising consisting essentially of or consisting of a chymotrypsin inhibitor and a pharmaceutically acceptable carrier. The composition can be administered in combination with another agent for treatment of BPH, for example, a 5-alpha reductase inhibitor (e.g., finasteride) or an alpha adrenergic inhibitor (e.g., doxazosine).

Diarrhea is a common complication in HIV patients. It appears that diarrhea is particularly common in patients treated with protease inhibitors. Prolonged diarrhea impacts quality of life and can contribute to weight loss, malnutrition, immunosuppression, poor drug absorption, non-compliance with therapy and mortality. Certain HIV protease inhibitors also inhibit chymotrypsin. Thus, treatment with HIV protease inhibitors may reduce that action of chymotrypsin in the gastrointestinal tract. Reduced chymotrypsin activity can lead to increased levels of active guanylin. Since guanylin promotes intestinal motility, this increase in active guanylin can lead to diarrhea and other gastrointestinal disorders. Thus, the invention features methods for treating or preventing diarrhea and/or other gastrointestinal disorders in patients, particularly HIV patients and others being treated with a protease inhibitor that inhibits chymotrypsin, by administering to the patient chymotrypsin. The gastrointestinal side-effects of HIV protease inhibitors can be mitigated by modifying the inhibitors to have reduced chymotrypsin inhibition activity. Thus, the invention features a method for identifying protease inhibitors, e.g., HIV protease inhibitors, having reduced gastrointestinal side effects. The methods entail testing a known or candidate protease inhibitor for its ability to inhibit chymotrypsin and selecting a known or candidate protease inhibitor having reduced chymotrypsin inhibition and retaining the ability to inhibit HIV protease. The ability of a compound to inhibit HIV protease and the ability of a compound to inhibit chymotrypsin can be assessed using standard *in vitro* assays.

The invention also feature compounds that are analogues of an HIV protease inhibitor and have reduced chymotrypsin inhibition activity while retaining the ability to inhibit HIV protease.

The details of one or more embodiments of the invention are set forth in the accompanying description and claims. The publications and patents referenced herein are incorporated by reference.

FIGURES

Figure 1 shows the results of a T84 cGMP assay to assess guanylin activity after a chymotrypsin digestion assay. The chymotrypsin digestion assay was performed in the presence or absence of chymostatin.

Figure 2 shows the results of LC/MS analysis of the processing of guanylin in a chymotrypsin digestion assay. The chymotrypsin digestion assay was performed in the presence or absence of chymostatin.

DETAILED DESCRIPTION

Guanylin binds to and activates the guanylate cyclase (GC-C) receptor, a key regulator of fluid and electrolyte balance in the intestine and kidney. When stimulated, this receptor, which is located on the apical membrane of the intestinal epithelial surface, causes an increase in intestinal epithelial cyclic GMP (cGMP). This increase in cGMP is believed to cause a decrease in water and sodium absorption and an increase in chloride and potassium ion secretion, leading to changes in intestinal fluid and electrolyte transport and increased intestinal motility. The intestinal GC-C receptor possesses an extracellular ligand binding region, a transmembrane region, an intracellular protein kinase-like region and a cyclase catalytic domain. Proposed functions for the GC-C receptor are fluid and electrolyte homeostasis, the regulation of epithelial cell proliferation and the induction of apoptosis (Shaibhubhai 2002 *Curr Opin Drug Dis Devel* 5:261-268).

In the human body an inactive form of chymotrypsin, chymotrypsinogen is produced in the pancreas. When this inactive enzyme reaches the small intestine it is converted to active chymotrypsin by the excision of two di-peptides. Active chymotrypsin will cleave peptides at the peptide bond on the carboxy-terminal side of Trp, Tyr or Phe and can cleave a peptide at the peptide bond on the amino terminal side of a Leu, Ile or Val (and, at elevated

pH, His). Thus, chymotrypsin can cleave guanylin as shown by Greenberg et al. (J Investig Med 45:276-82, 1997). Chymotrypsin-mediated inactivation of guanylin can be prevented by chymostatin, a chymotrypsin inhibitor.

Chymotrypsin Inhibitors

A wide variety of peptide and non-peptide chymotrypsin inhibitors are known. For example, alpha-2 antiplasmin (Potempa et al. 1988 Science 241: 699-700), members of the alpha-1 antichymotrypsin family (Forsyth et al. 2003 Genomics 81: 336-45), gelin (U.S. 5,397,694), hirustatin (Sollner et al. 1994 Eur J Biochem. 219: 937-43), and certain eglins (Seemuller et al. 1981 Methods Enzymol. 804-816) are peptide inhibitors of chymotrypsin. In addition, a large number of peptide inhibitors of chymotrypsin are reviewed by Schoofs et al. (2002 Curr Pharm Des. 8: 483-91) and by Salier et al. (1996 Biochem J. 315: 1-9). Synthetic peptide inhibitors of chymostrypsin are known (see, e.g., JP 4013698 A2 and JP 04013697 A2). In addition, various small molecule inhibitors of chymotrypsin have been described (see, e.g., EP 0071433; JP 56092217 A2; U.S. 4,755,383; U.S. 4,639,435; EP 0128007; U.S. 4,898,876; U.S. 4,620,005; U.S. 4,605,739; Rizzi et al. 1992 Biochem Int. 28:385-92; Luisetti et al. 1989 Biochem Biophys Res Commun. 165:568-73; Yokoo et al 1987 Yakugaku Zasshi 107:732-7; Boulanger 1986 Journal of Medicinal Chemistry 29:1483-7; McBride et al. 1996 J Mol Biol. 259: 819-27; McBride et al. 2000 J Pept Sci. 6:446-52). These small molecule inhibitors include: 3-[2-(2-thiophencarboxythio)]-propanoyl-4-thioazolidin carboxylic acid (YS3025) (Rizzi et al., supra) and MR889 (Luisetti et al., supra). A number of chymotrypsin inhibitors are available from commercial suppliers, including: chymostatin, aprotinin, N-tosyl-L-phenylalaninechloromethyl ketone, 4-(2-aminoethyl)-benzenesulfonylfluoride hydrochloride, benzamidine, di-isopropyl phosphofluoridate, 6-aminocaproic acid, CAS Registry No. 88070-98-8, CAS Registry No. 87928-05-0. A number of chymotrypsin inhibitors are produced by plants, including: C12 (Longstaff et al 1990 Biochemistry 29:7339-47), CI-2A (U.S. 5,167,483), CI-2A (WO 9205239), WCI-3 (Shibata et al. 1988 J Biochem (Tokyo) 104:537-43) WCI-2 (Habu et al. 1992 J Biochem (Tokyo) 111:249-58), and WCI-x (Habu et al., supra). Other plant-derived inhibitors have also been described (Bryant et al. 1976 Biochemistry 15:3418-24; Hass et al. 1982

Biochemistry 21:752-6; Birk 1985 Int J Pept Protein Res. 25:113-31; and Kollipara et al. 1992 Journal of Agricultural and Food Chemistry 40:2356-63). These chymotrypsin inhibitors and others are useful in the methods of the invention.

Certain HIV protease inhibitors may inhibit chymotrypsin and may undesirably potentiate the activity of guanylin. Among these inhibitors are: ritonavir (André et al. 1998 Proc Natl Acad Sci U S A 95:13120-4 and Schmidtke et al. 1999 J Biol Chem 274:35734-40), saquinavir (Hosseini et al. 2001 J Neuroimmunol. 118:233-44) and indinavir/indavir (Piccinini et al. 2002 AIDS 16:693-700). These HIV protease inhibitors and others have the ability to inhibit chymotrypsin and can cause gastrointestinal side-effects. To reduce these side-effects, a patient being treated with such an inhibitor can also be treated with guanylin or an analogue or variant of guanylin.

Chymotrypsin Activity Assays

Any standard chymotrypsin activity assay can be used to assess chymotrypsin inhibitors and to identify HIV protease inhibitors having reduced chymotrypsin inhibition.

For example chymotrypsin activity can be measured using N-Glutaryl-Lphenylalanine p-nitroanilide (Sigma-Aldrich, Inc; Catalog No. 49738) as a substrate and cc-Chymotrypsin from bovine pancreas (EC 3 2 1. 1; Sigma-Aldrich; Catalog No. C4129) in an assay described by Kakade et al. (Cereal Chemistry, 51. 376 (1974)). In this assay, chymotrypsin hydrolyzes the substrate N-Glutaryl-L-phenylalanine-p-nitroanilide present in excess. The release of p-nitroanilide, a yellow dye, is measured spectrophotometrically.

It has been suggested that the chymotrypsin-like activity of the 20S proteasome is inhibited by HIV protease inhibitors. Thus, it can be desirable to assess chymotrypsin inhibitors and HIV protease inhibitors using an assay that measures the chymotrypsin-like activity of the proteasome. Such an assay is described by Elliott et al. (Methods in Molecular Medicine 85: 163-172, 2003). This assay can be used to accurately determine the level of proteasome activity in rodent blood samples. The assay entails measuring

proteasome activity at either or both of the two proteolytic sites (chymotryptic and tryptic) within the 20S core of the proteasome and determining degree of inhibition conferred by a test agent.

An additional chymotrypsin assay is described by Kourteva et al. (Analytical Biochemistry 162:345-9, 1987) This assays is rapid and particularly useful for assessing higher molecular weight inhibitors. Briefly, a test compound is spotted onto an agar film which contains TLCK-chymotrypsin. Enzyme inhibition is visualized as colorless zones on a pink background after the films are stained with a chromogenic substrate N-acetyl-DL-phenylalanine-β-naphthyl ester. A variation on this assay can be use to assess trypsin activity and this can be useful for assessing the selectivity of an inhibitor.

Guanylin Degradation Assay

Since guanylin is susceptible to digestion by chymotrypsin, chymotrypsin activity can be assayed based on guanylin cleavage. Guanylin (Sigma, G-116) or a guanylin variant were resuspended in 5ml of 100mM Tris-HCl, 2mM $CaCl_2$, pH 7.8 at 30°C for a final concentration of 0.01mg/ml. From this stock, six (6) 500μl aliquots were prepared in 2 ml Eppendorf tubes and labeled "Control", "T0", "T15", "T30", "T60", and "T180", with "T_" representing timepoints, in minutes. 5μl of a 10mM chymostatin (Sigma, C7268) stock (5mg of chymostatin resuspended in 824μl of DMSO) was added to the "Control" samples and all samples were incubated for 5 minutes at 30°C. Chymostatin inhibits chymotrypsin activity and this sample served as a negative control. HIV protease inhibitor derivatives to be tested for chymotrypsin inhibition activity were added to experimental samples. Next, 20μl of a chymotrypsin (Sigma, C6423) enzyme solution (0.01mg/ml bovine chymotrypsin enzyme in 1mM HCl, 2mM Calcium Chloride) were added to the samples and mixed by inversion. Samples are incubated at 30°C. The "T0"samples were collected at time=0 minutes by adding 5ul of a 10mM chymostatin stock and subsequently stored at -80°C. All other timepoint samples are taken in a similar manner, with the "Control" samples collected parallel to the time=180 minutes samples. Determination of sensitivity to digestion by

chymotrypsin was determined by LCMS analysis and by *in vitro* activity in the T84 cGMP assays described below.

For LC/MS analysis, samples were injected (10 μL) onto a reverse phase HPLC column (Waters Atlantis dC_{18} 1.0 x 150mm, 3 um particle size, 186001283) at 20°C, and were eluted with a reverse phase gradient (Mobile Phase A: 5mM NH_4OAc in dH_2O, 0.1% formic acid, Mobile Phase B: 5mM NH_4OAc in 80% methanol and 20% dH_2O, 0.1% formic acid; Initial condition of 5% B, ramping to 95% B over 35 minutes, and holding for 3 minutes, then returning to initial conditions over the next 7 minutes, all at a flow rate of 0.07 mL/min.). At 45 minutes, the gradient was at initial conditions of 5%B and held for 15 minutes. Guanylin samples were detected by quadrapole-time of flight mass spectrometry in TOF scan mode (cone voltage = 30 V; collision =4 eV). Chymotrypsin sensitivity was determined by the loss of the initial mass species and the formation of the product mass species, with respect to time. Instrument response was converted into percentage units by comparison of the response of the initial mass versus the product mass, with "T0" representing total response of initial mass for all samples.

Activation of the intestinal GC-C receptor by guanylin (T84 cGMP assay)

The ability of guanylin to activate the intestinal GC-C receptor was assessed in an assay employing the T84 human colon carcinoma cell line (American Type Culture Collection (Bethesda, Md.). For the assays cells were grown to confluency in 24-well culture plates with a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum and were used at between passages 54 and 60.

Briefly, monolayers of T84 cells in 24-well plates were washed twice with 1 ml/well DMEM, then incubated at 37°C for 10 min with 0.45 ml DMEM containing 1 mM isobutylmethylxanthine (IBMX), a cyclic nucleotide phosphodiesterase inhibitor. Test peptides (50μl) were then added and incubated for 30 minutes at 37°C. The media was aspirated and the reaction was then terminated by the addition of ice cold 0.5 ml of 0.1N

HCl. The samples were held on ice for 20 minutes and then evaporated to dryness using a heat gun or vacuum centrifugation. The dried samples were resuspended in 0.5ml of phosphate buffer provided in the Cayman Chemical Cyclic GMP EIA kit (Cayman Chemical, Ann Arbor, MI). Cyclic GMP was measured by EIA according to procedures outlined in the Cayman Chemical Cyclic GMP EIA kit. Figure 1 shows that guanylin stimulated cGMP activity decreases over time in the presence of chymotrypsin. This activity was retained when the chymotrypsin inhibitor, chymostatin was present. Figure 2 shows the results of LC/MS analysis of the processing of guanylin in the chymotrypsin digestion assay.

Intestinal GC-C receptor binding assay

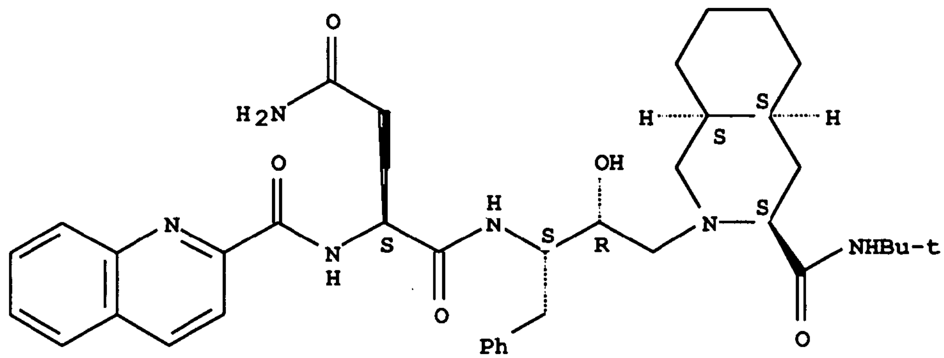
The ability of peptides and other agents to bind to the intestinal GC-C receptor can be tested as follows. Cells of the T84 human colon carcinoma cell line (American Type Culture Collection (Bethesda, Md.) are grown to confluence in 24-well culture plates with a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum. Cells used in the assay are typically between passages 54-60. Briefly, T84 cell monolayers in 24-well plates are washed twice with 1 ml of binding buffer (DMEM containing 0.05% bovine serum albumin and 25 mM HEPES, pH 7.2), then incubated for 30 min at 37°C in the presence of mature radioactively labeled *E. coli* ST peptide and the test material at various concentrations. The cells are then washed four times with 1 ml of DMEM and solubilized with 0.5 ml/well 1N NaOH. The level of radioactivity in the solubilized material is then determined using standard methods.
In some cases, intestinal epithelial cell preparations may be used instead of T84 cells to assess receptor binding.

Modified HIV Protease Inhibitors with Reduced Chymotrypsin Inhibition

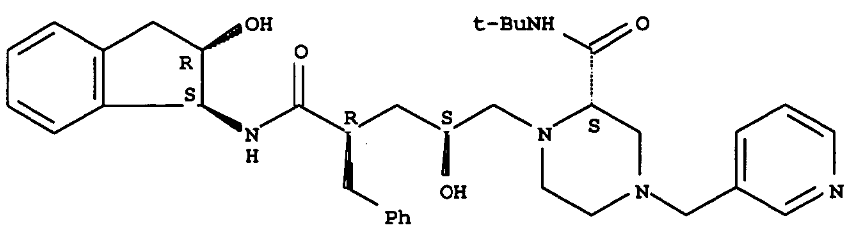
An HIV protease inhibitor variant having reduced chymotrypsin inhibition will be less likely to potentiate the action of guanylin. Thus, such inhibitors are less likely to be associated with gastrointestinal side-effects. HIV protease inhibitors such as those depicted

below can be modified to reduce chymotrypsin inhibition. The modification can include replacing a hydroxyl group with a phosphonate group or with an ester (alkyl, aralkyl, heteroaralkyl).

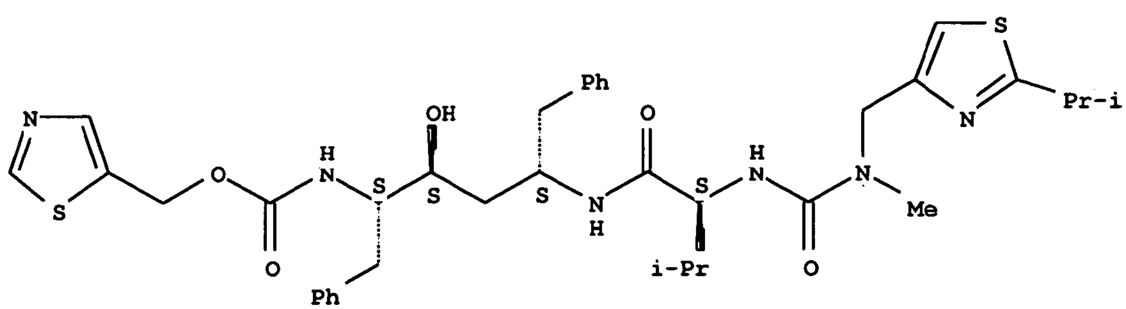
Saquinavir (CAS Registry No. 127779-20-8)



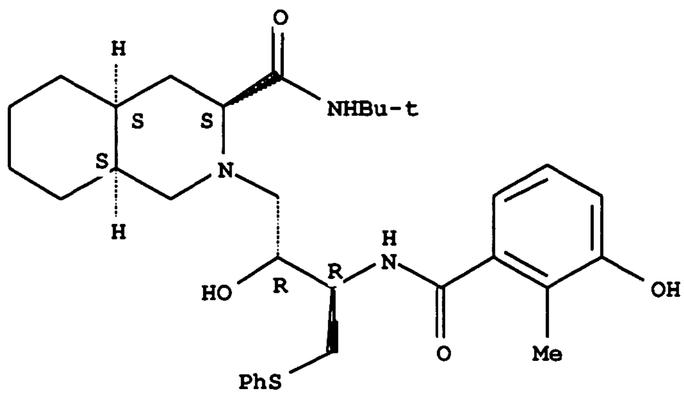
Indavir (CAS Registry No 150378-17-9)



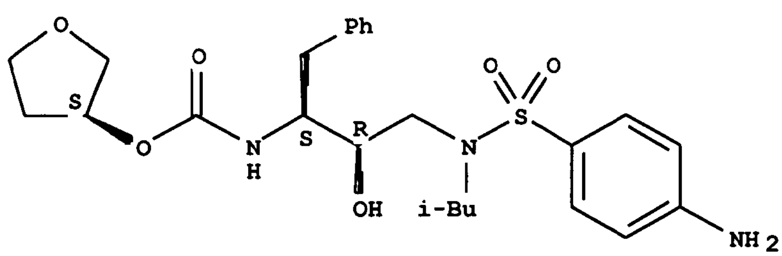
Norvir (CAS Registry No. 155213-67-5)



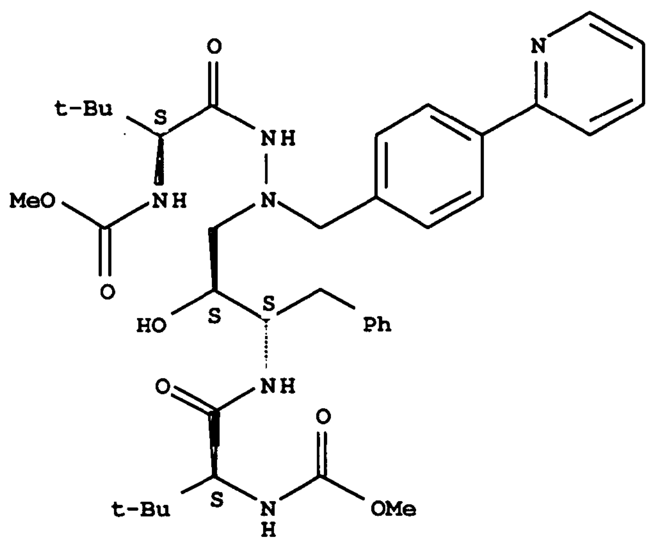
Nelfinavir (CAS Registry No. 159989-64-7)



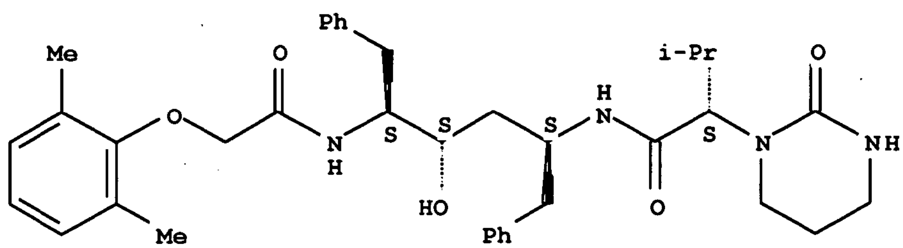
Amprinavir (CAS Registry No. 161814-49-9)



Atazanivir (CAS Registry No. 198904-31-3)



Lopinavir (CAS Registry No. 369372-47-4)



Murine gastrointestinal transit (GIT) assay

In order to determine if a chymotrypsin inhibitor or an HIV protease inhibitor has an effect on intestinal motility, the inhibitor be tested in the murine gastrointestinal transit (GIT) assay (Moon et al. *Infection and Immunity* 25:127, 1979). This assay can also be used to determine if guanylin or a guanylin analogue or variant administered with an HIV protease inhibitor has a beneficial effect on intestinal motility and to examine the effect of a chymotrypsin inhibitor on intestinal motility. In this assay, charcoal, which can be readily

visualized in the gastrointestinal tract is administered to mice after the administration of a test compound. The distance traveled by the charcoal is measured and expressed as a percentage of the total length of the colon.

Mice are fasted with free access to water for 12 to 16 hours before the treatment with peptide or control buffer. A test agent is orally administered in buffer (20mM Tris pH 7.5) seven minutes before being given an oral dose of 5% Activated Carbon (Aldrich 242276-250G). Control mice are administered buffer only before being given a dose of Activated Carbon. After 15 minutes, the mice are sacrificed and their intestines from the stomach to the cecum are dissected. The total length of the intestine as well as the distance traveled from the stomach to the charcoal front is measured for each animal and the results are expressed as the percent of the total length of the intestine traveled by the charcoal front. Results are reported as the average of 10 mice ± standard deviation. A comparison of the distance traveled by the charcoal between the mice treated with the agent versus the mice treated with vehicle alone is performed using a Student's t test and a statistically significant difference is considered for $P<0.05$. Positive controls for this assay may include and Zelnorm®, a drug approved for IBS that is an agonist for the serotonin receptor 5HT4.

Suckling mouse model of intestinal secretion (SuMi assay)

Chymotrypsin inhibitors and other agents can be tested for their ability to increase intestinal secretion using a suckling mouse model of intestinal secretion. In this model a test compound is administered to suckling mice that are between seven and nine days old. After the mice are sacrificed, the gastrointestinal tract from the stomach to the cecum is dissected ("guts"). The remains ("carcass") as well as the guts are weighed and the ratio of guts to carcass weight is calculated. If the ratio is above 0.09, one can conclude that the test compound increases intestinal secretion. Controls for this assay may include Zelnorm®

Phenylbenzoquinone-induced writhing model

The PBQ-induced writhing model can be used to assess whether administration of a compound such as a chymotrypsin inhibitor or a chymotrypsin inhibitor administered with

guanylin or a guanylin analogue reduces pain. This model is described by Siegmund et al. (1957 Proc. Soc. Exp. Bio. Med. 95:729-731). Briefly, one hour after oral dosing with a test compound, morphine or vehicle, 0.02% phenylbenzoquinone (PBQ) solution (12.5 mL/kg) is injected by intraperitoneal route into the mouse. The number of stretches and writhings are recorded from the 5th to the 10th minute after PBQ injection, and can also be counted between the 35th and 40th minute and between the 60th and 65th minute to provide a kinetic assessment. The results are expressed as the number of stretches and writhings (mean ± SEM) and the percentage of variation of the nociceptive threshold calculated from the mean value of the vehicle-treated group. The statistical significance of any differences between the treated groups and the control group is determined by a Dunnett's test using the residual variance after a one-way analysis of variance ($P< 0.05$) using SigmaStat Software.

Colonic hyperalgesia animal models

Hypersensitivity to colorectal distension is a common feature in patients with IBS and may be responsible for the major symptom of pain. Both inflammatory and non-inflammatory animal models of visceral hyperalgesia to distension have been developed to investigate the effect of compounds on visceral pain in IBS and can be used to assess the impact of a compound such as a chymotrypsin inhibitor or a chymotrypsin inhibitor administered with guanylin or a guanylin analogue.

I. Trinitrobenzenesulphonic acid (TNBS)-induced rectal allodynia model

Male Wistar rats (220-250 g) are premedicated with 0.5 mg/kg of acepromazine injected intraperitoneally (IP) and anesthetized by intramuscular administration of 100 mg/kg of ketamine. Pairs of nichrome wire electrodes (60 cm in length and 80 μm in diameter) are implanted in the striated muscle of the abdomen, 2 cm laterally from the white line. The free ends of electrodes are exteriorized on the back of the neck and protected by a plastic tube attached to the skin. Electromyographic (EMG) recordings are started 5 days after surgery. Electrical activity of abdominal striated muscle is recorded with an electroencephalograph

machine (Mini VIII, Alvar, Paris, France) using a short time constant (0.03 sec.) to remove low-frequency signals (<3 Hz).

Ten days post surgical implantation, trinitrobenzenesulphonic acid (TNBS) is administered to induce rectal inflammation. TNBS (80 mg kg^{-1} in 0.3 ml 50 % ethanol) is administered intrarectally through a silicone rubber catheter introduced at 3 cm from the anus under light diethyl-ether anesthesia, as described (Morteau et al. 1994 Dig Dis Sci 39:1239). Following TNBS administration, rats are placed in plastic tunnels where they are severely limited in mobility for several days before colorectal distension (CRD). Experimental compound is administered one hour before CRDwhich is performed by insertion into the rectum, at 1 cm of the anus, a 4 cm long balloon made from a latex condom (Gue et al, 1997 *Neurogastroenterol. Motil.* 9:271). The balloon is fixed on a rigid catheter taken from an embolectomy probe (Fogarty). The catheter attached balloon is fixed at the base of the tail. The balloon, connected to a barostat is inflated progressively by step of 15 mmHg, from 0 to 60 mmHg, each step of inflation lasting 5 min. Evaluation of rectal sensitivity, as measured by EMG, is performed before (1-2 days) and 3 days following rectal instillation of TNBS.

The number of spike bursts that corresponds to abdominal contractions is determined per 5 min periods. Statistical analysis of the number of abdominal contractions and evaluation of the dose-effects relationships is performed by a one way analysis of variance (ANOVA) followed by a post-hoc (Student or Dunnett tests) and regression analysis for ED50 if appropriate.

II. <u>Stress-induced hyperalgesia model</u>

Male Wistar Rats (200-250 g) are surgically implanted with nichrome wire electrodes as in the TNBS model. Ten days post surgical implantation, partial restraint stress (PRS), is performed as described by Williams et al. for two hours (Williams et al. 1988 Gastroenterology 64:611). Briefly, under light anaesthesia with ethyl-ether, the foreshoulders, upper forelimbs and thoracic trunk are wrapped in a confining harness of paper tape to restrict, but not prevent body movements. Control sham-stress animals are

anaesthetized but not wrapped. Thirty minutes before the end of the PRS session, the animals are administered test-compound or vehicle. Thirty minutes to one hour after PRS completion, the CRD distension procedure is performed as described above for the TNBS model with barostat at pressures of 15, 30, 45 and 60mm Hg. Statistical analysis on the number of bursts is determined and analyzed as in the TNBS model above.

Administration of Chymotrypsin Inhibitors

For treatment of gastrointestinal disorders, chymotrypsin inhibitors can be administered orally, e.g., as a tablet, gel, paste, slurry, liquid, powder or in some other form. Orally administered compositions can include binders, flavoring agents, and humectants. The chymotrypsin inhibitors can also be administered rectally, e.g., by suppository. The chymotrypsin inhibitors can be co-administered with other agents used to treat gastrointestinal disorders including but not limited to acid suppressing agents such as Histamine-2 receptor agonists (H2As) and proton pump inhibitors (PPIs). The chymotrypsin inhibitor can be administered together with guanylin or a guanylin variant or analogue. The inhibitors can also be administered by rectal suppository. For the treatment of disorders outside the gastrointestinal tract such as congestive heart failure and benign prostatic hypertrophy, inhibitors are preferably administered parenterally or orally.

Chymotrypsin inhibitors preferably reach the small and/or large intestine in order to effectively reduce the activity of chymotrypsin that proteolytically digests guanylin. If the inhibitor(s) is to be administered orally, it is preferably formulated with an enteric coating. For example, the formulation can be provided with a non-porous, gastric acid-resistant polymer coating, e.g., a coating that is insoluble or only slightly soluble at pH 1.5 to pH 5, but is soluble above pH 5 or pH 5.5 up to or above pH 9. The polymer can include, for example, hydroxypropyl methyl cellulose phthalate, cellulose acetate phthalate, diethyl phthalate, dibutyl phthalate, and acrylic based polymers. The formulation can also be buffered by inclusion of a buffering agent, for example, sodium bicarbonate, potassium carbonate, potassium bicarbonate, ammonium carbonate, tromethamine, di(tris)hydroxymethylaminomethane) carbonate, tris-glycine, di-arginine, tri-arginine, poly-arginine, di-lysine, tri-lysine, poly-lysine, diethylamine and triethanolamine. It can be desirable for the buffering agent to provide a pH of from about 7 to about 9 in the small intestine or large intestine of a human patient. The formulation can also include a disintegrant, e.g., ursodiol, starch, modified starches, microcrystalline cellulose and propylene glycol alginate.

The chymotrypsin inhibitors described herein can be used alone or in combination with other agents. For example, they can be administered together with an agent for treating a gastrointestinal disorder. The chymotrypsin inhibitors can be administered in a combination therapy with guanylin itself or a guanylin analogue or variant.

Combination therapy can be achieved by administering two or more agents, e.g., a chymotrypsin inhibitor and an agent for treating a gastrointestinal disorder or an analgesic peptide or compound, each of which is formulated and administered separately, or by administering two or more agents in a single formulation. Other combinations are also encompassed by combination therapy. For example, two agents can be formulated together and administered in conjunction with a separate formulation containing a third agent. While the two or more agents in the combination therapy can be administered simultaneously, they need not be. For example, administration of a first agent (or combination of agents) can precede administration of a second agent (or combination of agents) by minutes, hours, days,

or weeks. Thus, the two or more agents can be administered within minutes of each other or within 1, 2, 3, 6, 9, 12, 15, 18, or 24 hours of each other or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 days of each other or within 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks of each other. In some cases even longer intervals are possible. While in many cases it is desirable that the two or more agents used in a combination therapy be present in within the patient's body at the same time, this need not be so.

Combination therapy can also include two or more administrations of one or more of the agents used in the combination. For example, if agent X and agent Y are used in a combination, one could administer them sequentially in any combination one or more times, e.g., in the order X-Y-X, X-X-Y, Y-X-Y, Y-Y-X, X-X-Y-Y, etc.

The agents, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, etc.

The agents can be administered, e.g., by intravenous injection, intramuscular injection, subcutaneous injection, or by other routes. The agents can be administered orally, e.g., as a tablet, gel, paste, slurry, liquid, powder or in some other form. Orally administered compositions can include binders, flavoring agents, and humectants. The agents can be included in dentifrices or oral washes. Thus, oral formulations can include abrasives and foaming agents. The agents can also be administered transdermally or in the form of a suppository.

The agents can be a free acid or base, or a pharmacologically acceptable salt thereof. Solids can be dissolved or dispersed immediately prior to administration or earlier. In some circumstances the preparations include a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injection can include sterile aqueous or organic solutions or dispersions which include, e.g., water, an alcohol, an organic solvent,

an oil or other solvent or dispersant (e.g., glycerol, propylene glycol, polyethylene glycol, and vegetable oils). Pharmaceutical agents can be sterilized by filter sterilization or by other suitable means.

Suitable pharmaceutical compositions in accordance with the invention will generally include an amount of the active compound(s) with an acceptable pharmaceutical diluent or excipient, such as a sterile aqueous solution, to give a range of final concentrations, depending on the intended use. The techniques of preparation are generally well known in the art, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, 1995.

The agents described herein and combination therapy agents can be packaged as a kit that includes single or multiple doses of two or more agents, each packaged or formulated individually, or single or multiple doses of two or more agents packaged or formulated in combination. Thus, one or more agents can be present in first container, and the kit can optionally include one or more agents in a second container. The container or containers are placed within a package, and the package can optionally include administration or dosage instructions. A kit can include additional components such as syringes or other means for administering the agents as well as diluents or other means for formulation.

Analgesic Agents

The chymotrypsin inhibitors described herein can be used in combination therapy with an analgesic agent, e.g., an analgesic compound or an analgesic peptide. The analgesic agent can optionally be covalently attached to a peptide described herein. Among the useful analgesic agents are: Ca channel blockers, 5HT receptor antagonists (for example 5HT3, 5HT4 and 5HT1 receptor antagonists), opioid receptor agonists (loperamide, fedotozine, and fentanyl), NK1 receptor antagonists, CCK receptor agonists (e.g., loxiglumide), NK1 receptor antagonists, NK3 receptor antagonists, norepinephrine-serotonin reuptake inhibitors (NSRI), vanilloid and cannabanoid receptor agonists, and sialorphin. Analgesics agents in the various classes are described in the literature.

Among the useful analgesic peptides are sialorphin-related peptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO:), including: VQHNPR (SEQ ID NO:); VRQHNPR (SEQ ID NO:); VRGQHNPR (SEQ ID NO:); VRGPQHNPR (SEQ ID NO:); VRGPRQHNPR (SEQ ID NO:); VRGPRRQHNPR (SEQ ID NO:); and RQHNPR (SEQ ID NO:). Sialorphin-related peptides bind to neprilysin and inhibit neprilysin-mediated breakdown of substance P and Met-enkephalin. Thus, compounds or peptides that are inhibitors of neprilysin are useful analgesic agents which can be administered with the peptides of the invention in a co-therapy or linked to the peptides of the invention, e.g., by a covalent bond. Sialophin and related peptides are described in U.S. Patent 6,589,750; U.S. 20030078200 A1; and WO 02/051435 A2.

Opioid receptor antagonists and agonists can be administered with the peptides of the invention in co-therapy or linked to the peptide of the invention, e.g., by a covalent bond. For example, opioid receptor antagonists such as naloxone, naltrexone, methyl nalozone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, and nor-binaltorphimine are thought to be useful in the treatment of IBS. It can be useful to formulate opioid antagonists of this type is a delayed and sustained release formulation such that initial release of the antagonist is in the mid to distal small intestine and/or ascending colon. Such antagonists are described in WO 01/32180 A2. Enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-homoserine) is an agonist of the mu and delta opioid receptors and is thought to be useful for increasing intestinal motility (*Eur. J. Pharm.* 219:445, 1992), and this peptide can be used in conjunction with the peptides of the invention. Also useful is trimebutine which is thought to bind to mu/delta/kappa opioid receptors and activate release of motilin and modulate the release of gastrin, vasoactive intestinal peptide, gastrin and glucagons. Kappa opioid receptor agonists such as fedotozine, ketocyclazocine, and compounds described in WO 03/097051 A2 can be used with or linked to the peptides of the invention. In addition, mu opioid receptor agonists such as morphine, diphenyloxylate, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH_2; WO 01/019849 A1) and loperamide can be used.

Tyr-Arg (kyotorphin) is a dipeptide that acts by stimulating the release of met-enkephalins to elicit an analgesic effect (*J. Biol. Chem* 262:8165, 1987). Kyotorphin can be used with or linked to the peptides of the invention.

CCK receptor agonists such as caerulein from amphibians and other species are useful analgesic agents that can be used with or linked to the peptides of the invention.

Conotoxin peptides represent a large class of analgesic peptides that act at voltage gated Ca channels, NMDA receptors or nicotinic receptors. These peptides can be used with or linked to the peptides of the invention.

Peptide analogs of thymulin (FR Application 2830451) can have analgesic activity and can be used with or linked to the peptides of the invention.

CCK (CCKa or CCKb) receptor antagonists, including loxiglumide and dexloxiglumide (the R-isomer of loxiglumide) (WO 88/05774) can have analgesic activity and can be used with or linked to the peptides of the invention.

Other useful analgesic agents include 5-HT4 agonists such as tegaserod/zelnorm and lirexapride. Such agonists are described in: EP1321142 A1, WO 03/053432A1, EP 505322 A1, EP 505322 B1, US 5,510,353, EP 507672 A1, EP 507672 B1, and US 5,273,983.

Calcium channel blockers such as ziconotide and related compounds described in, for example, EP625162B1, US 5,364,842, US 5,587,454, US 5,824,645, US 5,859,186, US 5,994,305, US 6,087,091, US 6,136,786, WO 93/13128 A1, EP 1336409 A1, EP 835126 A1, EP 835126 B1, US 5,795,864, US 5,891,849, US 6,054,429, WO 97/01351 A1, can be used with or linked to the peptides of the invention.

Various antagonists of the NK-1, NK-2, and NK-3 receptors (for a review see Giardina et al. 2003 *Drugs* 6:758) can be can be used with or linked to the peptides of the invention.

NK1 receptor antagonists such as: aprepitant (Merck & Co Inc), vofopitant, ezlopitant (Pfizer, Inc.), R-673 (Hoffmann-La Roche Ltd), SR-14033 and related compounds described in, for example, EP 873753 A1, US 20010006972 A1, US 20030109417 A1, WO 01/52844 A1, can be used with or linked to the peptides of the invention.

NK-2 receptor antagonists such as nepadutant (Menarini Ricerche SpA), saredutant (Sanofi-Synthelabo), SR-144190 (Sanofi-Synthelabo) and UK-290795 (Pfizer Inc) can be used with or linked to the peptides of the invention.

NK3 receptor antagonists such as osanetant (Sanofi-Synthelabo), talnetant and related compounds described in, for example, WO 02/094187 A2, EP 876347 A1, WO 97/21680 A1, US 6,277,862, WO 98/11090, WO 95/28418, WO 97/19927, and Boden et al. (*J Med Chem.* 39:1664-75, 1996) can be used with or linked to the peptides of the invention.

Norepinephrine-serotonin reuptake inhibitors such as milnacipran and related compounds described in WO 03/077897 A1 can be used with or linked to the peptides of the invention.

Vanilloid receptor antagonists such as arvanil and related compounds described in WO 01/64212 A1 can be used with or linked to the peptides of the invention.

Where the analgesic is a peptide and is covalently linked to a peptide described herein the resulting peptide may also include at least one trypsin or chymotrypsin cleavage site. When present within the peptide, the analgesic peptide may be preceded by (if it is at the carboxy terminus) or followed by (if it is at the amino terminus) a chymotrypsin or trypsin cleavage site that allows release of the analgesic peptide.

In addition to sialorphin-related peptides, analgesic peptides include: AspPhe, endomorphin-1, endomorphin-2, nocistatin, dalargin, lupron, zicnotide, and substance P.

Methods of Treatment

The inhibitors of the invention can be used for the treatment or prevention of cancer, pre-cancerous growths, or metastatic growths. For example, they can be used for the prevention or treatment of: colorectal/local metastasized colorectal cancer, gastrointestinal tract cancer, lung cancer, cancer or pre-cancerous growths or metastatic growths of epithelial cells, polyps, breast, colorectal, lung, ovarian, pancreatic, prostatic, renal, stomach, bladder, liver, esophageal and testicular carcinoma, carcinoma (e.g., basal cell, basosquamous, Brown-Pearce, ductal carcinoma, Ehrlich tumor, Krebs, Merkel cell, small or non-small cell lung, oat cell, papillary, bronchiolar, squamous cell, transitional cell, Walker), leukemia (e.g., B-cell, T-cell, HTLV, acute or chronic lymphocytic, mast cell, myeloid), histiocytonia, histiocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, plasmacytoma, reticuloendotheliosis, adenoma, adeno-carcinoma, adenofibroma, adenolymphoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, sclerosing angioma, angiomatosis, apudoma, branchionia, malignant carcinoid syndrome, carcinoid heart disease, carcinosarcoma, cementoma, cholangioma, cholesteatoma, chondrosarcoma, chondroblastoma, chondrosarcoma, chordoma, choristoma, craniopharyngioma, chrondrorna, cylindroma, cystadenocarcinoma, cystadenoma, cystosarconia phyllodes, dysgenninoma, ependymoma, Ewing sarcoma, fibroma, fibrosarcoma, giant cell tumor, ganglioneuroma, glioblastoma, glomangioma, granulosa cell tumor, gynandroblastoma, hamartoma, hemangioendothelioma, hemangioma, hemangio-pericytoma, hemangiosarcoma, hepatoma, islet cell tumor, Kaposi sarcoma, leiomyoma, leiomyosarcoma, leukosarcoma, Leydig cell tumor, lipoma, liposarcoma, lymphaugioma, lymphangiomyoma, lymphangiosarcoma, medulloblastoma, meningioma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, neurilemmoma, neuroma, neuroblastoma, neuroepithelioma, neurofibroma, neurofibromatosis, odontoma, osteoma,, osteosarcoma, papilloma, paraganglioma, paraganglionia. nonchroinaffin, pinealoma, rhabdomyoma,

rhabdomyosarcoma, Sertoli cell tumor, teratoma, theca cell tumor, and other diseases in which cells have become dysplastic, immortalized, or transformed.

The inhibitors of the invention can be used for the treatment or prevention of: Familial Adenomatous Polyposis (FAP) (autosomal dominant syndrome) that precedes colon cancer, hereditary nonpolyposis colorectal cancer (HNPCC), and inherited autosomal dominant syndrome.

For treatment or prevention of cancer, pre-cancerous growths and metastatic growths, the inhibitors can be used in combination therapy with radiation or chemotherapeutic agents, an inhibitor of a cGMP-dependent phosphodiesterase or a selective cyclooxygenase-2 inhibitor. A number of selective cyclooxygenase-2 inhibitors are described in WO 02/062369.

The inhibitors can be for treatment or prevention of inflammation. Thus, they can be used alone or in combination with inhibitor of cGMP-dependent phosphodiesterase or a selective cyclooxygenase-2 inhibitor for treatment of: organ inflammation, IBD (e.g, Crohn's disease, ulcerative colitis), asthma, nephritis, hepatitis, pancreatitis, bronchitis, cystic fibrosis, ischemic bowel diseases, intestinal inflammations/allergies, coeliac disease, proctitis, eosnophilic gastroenteritis, mastocytosis, and other inflammatory disorders.

The inhibitors can also be used to treat or prevent insulin-related disorders, for example: II diabetes mellitus, hyperglycemia, obesity, disorders associated with disturbances in glucose or electrolyte transport and insulin secretion in cells, or endocrine disorders. They can be also used in insulin resistance treatment and post-surgical and non-post surgery decrease in insulin responsiveness.

The inhibitors can be used to prevent or treat respiratory disorders, including, inhalation, ventilation and mucus secretion disorders, pulmonary hypertension, chronic obstruction of vessels and airways, and irreversible obstructions of vessels and bronchi.

The inhibitors can be used in combination therapy with a phosphodiesterase inhibitor. Examples of such inhibitors can be found in US Patent No. 6,333,354.

The inhibitors can also be used to prevent or treat: retinopathy, nephropathy, diabetic angiopathy, and edema formation

The inhibitors can also be used to prevent or treat neurological disorders, for example, headache, anxiety, movement disorders, aggression, psychosis, seizures, panic attacks, hysteria, sleep disorders, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes, memory loss, and narcolepsy. They may also be used as a sedative.

The inhibitors and detectably labeled inhibitors can be used as markers to identify, detect, stage, or diagnosis diseases and conditions of small intestine, including:
Crohn's disease, colitis, inflammatory bowel disease, tumors, benign tumors, such as benign stromal tumors, adenoma, angioma, adenomatous (pedunculated and sessile) polyps, malignant, carcinoid tumors, endocrine cell tumors, lymphoma, adenocarcinoma, foregut, midgut, and hindgut carcinoma, gastroinstestinal stromal tumor (GIST), such as leiomyorna, cellular leiomyoma, leiomyoblastoma, and leiomyosarcoma, gastrointestinal autonomic nerve tumor, malabsorption syndromes, celiac diseases, diverticulosis, Meckel's diverticulum, colonic diverticula, megacolon, Hirschsprung's disease, irritable bowel syndrome, mesenteric ischemia, ischemic colitis, colorectal cancer, colonic polyposis, polyp syndrome, intestinal adenocarcinoma, Liddle syndrome, Brody myopathy, infantile convulsions, and choreoathetosis

The inhibitors can be conjugated to another molecule (e.g., a diagnostic or therapeutic molecule) to target cells bearing the ST receptor, e.g., cystic fibrosis lesions and specific cells lining the intestinal tract. Thus, they can be used to target radioactive moieties or therapeutic moieties to the intestine to aid in imaging and diagnosing or treating colorectal/metastasized or local colorectal cancer and to deliver normal copies of the p53 tumor suppressor gene to the intestinal tract.

The inhibitors can be used alone or in combination therapy to treat erectile dysfunction.

The inhibitors can be used alone or in combination therapy to treat inner ear disorders, e.g., to treat Meniere's disease, including symptoms of the disease such as vertigo, hearing loss, tinnitus, sensation of fullness in the ear, and to maintain fluid homeostasis in the inner ear.

The inhibitors can be used alone or in combination therapy to treat disorders associated with fluid and sodium retention, e.g., diseases of the electrolyte-water/electrolyte transport system withing the kidney, gut and urogenital system, congestive heart failure, hypertension, hypotension, liver cirrhosis, and nephrotic syndrome. In addition they can be used to facilitate diuresis or control intestinal fluid.

The inhibitors can be used alone or in combination therapy to treat disorders associated with bicarbonate secretion, e.g., Cystic Fibrosis.

The inhibitors can be used alone or in combination therapy to treat disorders associated with liver cell regeneration.

Figure 1. Guanylin chymotrypsin sensitivity as determined by cGMP assay

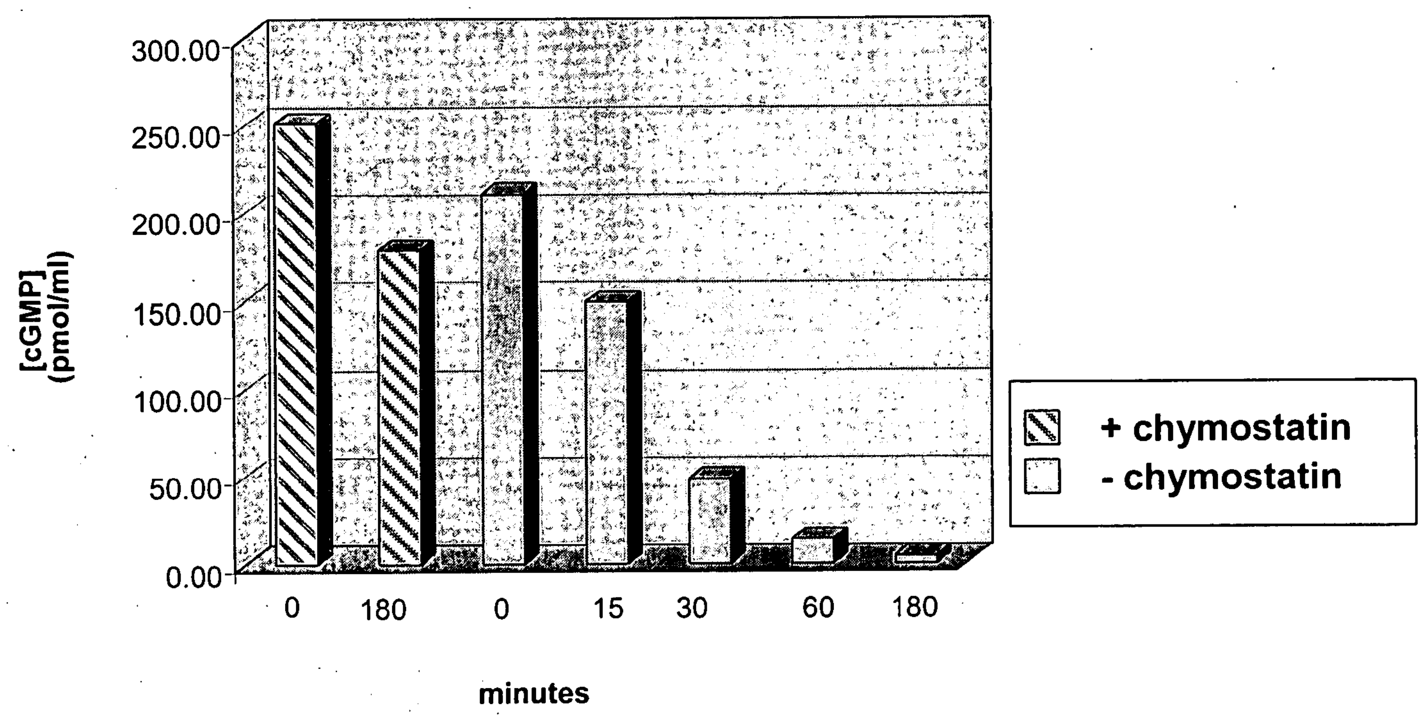


Figure 2. Guanylin chymotrypsin sensitivity as determined by LCMS analysis